PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Christine Markert-Hahn *et al.*
Application No.	:	10/647,720
Filed	:	August 25, 2003
For	:	METHOD FOR BISULFITE TREATMENT

Examiner	:	Joyce Tung
Art Unit	:	1637
Docket No.	:	810102.401

DECLARATION OF CHRISTINE MARKERT-HAHN, PH.D.
PURSUANT TO 37 C.F.R. §1.132

I, Christine Markert Hahn, Ph.D. declare as follows:

1. I currently hold the position of Manager of Pharmaceutical Biotech Production at Roche Diagnostics GmbH, located in Penzberg, Germany, a company affiliated with the assignee of the above-identified application (the "Application").

2. As evident from the enclosed CV, I have been involved in biochemical research since I commenced my biochemistry studies at Tübingen University, Germany, in 1980. I joined Roche in 1989, from 2002 to 2006 I pursued biochemical research in the field of molecular diagnostics, primarily in oncology. As part of my responsibilities, I have been involved in establishing and validating methods in the field of DNA methylation. I am also an inventor on a number of patents and patent applications (see enclosed list of patents and applications).

3. I co-invented the subject matter of the Application with Dr. Dirk Block. It is fair to say that I have a profound knowledge not only of the bisulfite method

and DNA methylation based diagnostic technology described in the Application, but of the knowledge available in this field as of the time of filing the Application.

4. I submit this Declaration as evidence of the non-obviousness of the presently claimed bisulfite reaction-based method over the references cited in this Office Action. I am familiar with the Application and have reviewed the outstanding final Office Action mailed on November 28, 2007, in addition to the documents cited by the Examiner in this Office Action. I provide my opinion on the invention as defined in the claims currently pending, taking into account the disclosure of the Application as originally filed, the general knowledge available in the art at the time the Application was filed, and the teachings from the below referenced prior art.

5. The present invention relates to an improved bisulfite reaction-based method for the conversion of cytosine bases in a nucleic acid to uracil bases. The presently claimed method is useful for determining methylation positions in a nucleic acid which, in turn, is of interest for diagnostic purposes in the field of epigenomics. The method is based, in pertinent part, on performing the deamination step, the desulfonation step or both on a solid phase-bound nucleic acid, such as a patient DNA. In particular, I submit that it was a surprising and unexpected finding of the present invention when we found out that single stranded DNA bound to a solid phase could actually be accessed successfully by the bisulfite ions. This unexpected finding allows the process to be automated and renders the results obtained by the claimed method much more precise and accurate than was known in the art before August 2003 (*see* Application as published, *e.g.*, section [0011], Examples).

6. By way of background, the bisulfite method as described by Hermann *et al.* (U.S. Patent No. 5,789,146) allows the specific conversion of unmethylated cytosines of DNA (in contrast to methylated cytosines) into uracil, thereby creating a modified DNA sequence that allows the skilled artisan to measure specifically either the unmethylated or the methylated DNA by methods relying on hybridization techniques (*e.g.*, direct hybridization with labeled probes, hybridization of primers with

subsequent polymerase chain reaction; see Hermann *et al.*, at column 3, line 35 to column 4, line 44, Examples). The bisulfite method described by Hermann *et al.* basically consists of three steps: (i) denaturation of double stranded DNA, (ii) deamination of cytosine by bisulfite treatment under defined conditions and (iii) desulfonation by using alkaline conditions so as to obtain uracil. Notably, unlike the present invention, the method of Hermann *et al.* specifies that all steps are performed in solution (*see, e.g.,* Hermann *et al.*, at column 3, line 35 to column 4, line 44, Examples). In addition, and contrary to the present invention, the procedure of Hermann *et al.* is cumbersome, time consuming and hardly applicable to routine diagnostic purposes, let alone suitable for automation.

7. The publication of Gagna (US 2003/0096273) describes the immobilization of un-denatured nucleic acids (mainly synthetic model nucleic acids) to a solid support by modifying at least one strand so as to allow the nucleic acid to be bound to the solid support (*see* Gagna, at sections [0008] to [0010] and [0023 to 0025]). The bound nucleic acid is then used mainly for functional assays where interactions between the bound nucleic acid exhibiting a proper three-dimensional structure and other molecules (proteins, antibodies, drugs) are analyzed to get further insight into regulation of gene expression and repair or the efficacy of potential drugs to block gene expression (see Gagna, at sections [0008] to [0010]).

8. For the following reasons, I submit that a person skilled in the art would not have considered combining the methods of Gagna with the methods of Hermann *et al.* in arriving at the present invention defined in the present claims.

8.1. First, Gagna relates to a different technical field than Hermann *et al.* and the present invention. For example, Gagna addresses immobilization of un-denatured nucleic acids so as to allow analysis of interaction between the solid-phase bound nucleic acid and other molecules (*see* Gagna, at sections [0008] to [0010]). In contrast, Hermann *et al.* (*see* Hermann *et al.*, at column 3, line 35 to column 4, line 44, Examples) and the present invention (*see* claims and

Examples) relate to the field of using denatured nucleic acids to be subjected to the bisulfite reaction so as to measure specifically either the unmethylated or the methylated DNA.

8.2. In addition, the method of Gagna requires that the solid-phase bound nucleic acid is un-denatured (*see* Gagna, at sections [0008] to [0010]), as the analysis of the interaction with other molecules would otherwise not be possible. This is in stark contrast to Hermann *et al.* (see Hermann *et al.*, at column 3, line 35 to column 4, line 44, Examples) which requires denatured nucleic acids. This difference taken alone renders the teachings of Hermann *et al.* and Gagna quite incompatible, and would have prevented me from combining the two documents when attempting to come up with bisulfite-reaction based method for the conversion of cytosine bases in a solid-phase bound nucleic acid to uracil bases that is amenable to automation in August 2003.

8.3. Gagna also requires the nucleic acid to be modified before binding can take place (*see* Gagna, at sections [0008] to [0010] and [0023] to [0025]), which, in comparison to the present invention, renders the method of Gagna more complex than the present invention. This also argues against considering Gagna when developing a solid-phase based method for the conversion of cytosine bases to uracil bases.

8.4. Finally, according to Gagna, the binding of the nucleic acid to the support is irreversible [*see* Gagna, at sections [0023] to [0025]), which is in contradiction to the solution offered by the present invention.

9. In view of the above, there are a number of serious obstacles and differences between Hermann *et al.* and Gagna that would have prevented a skilled worker from combining the teachings described therein. In addition, these differences would have made it necessary to carry out substantial adaptations that, in my opinion, would not have been obvious to the skilled worker. As further detailed below, I also

believe that a skilled worker at the time the Application was filed would have not reasonably expected that carrying out the required adaptations would have yielded a method allowing a bisulfite reaction to be carried out with solid-phase bound nucleic acids.

10. The publication of Olek (Nucl. Ac. Res. 1996, 24924, p. 5064-5066) does not describe direct binding of nucleic acids to a solid phase. Rather, Olek merely describes embedding nucleic acids in low-melting agarose matrix for subsequent chemical treatment (*see* Olek, abstract). Thus, Olek would not have been helpful at all to the skilled worker having knowledge of Hermann *et al.* and/or Gagna to arrive at our invention as set out in the claims.

11. In the publication of Weindel (WO 01/37291), the binding of nucleic acids to magnetic glass particles is performed solely with the intention to purify the nucleic acid from contaminating substances (*see, e.g.,* Weindel, at page 1, lines 1 to 6 and page 13, third paragraph), as opposed to subjecting the nucleic acid to any chemical reaction, as in the case of Hermann *et al.* or the present invention. Thus, a skilled worker would not have been motivated by Weindel to subject solid-phase bound nucleic acids to a chemical reaction, let alone a bisulfite reaction.

12. At the time the Application was filed, a person skilled in the art would not have reasonably expected that a denatured nucleic acid bound to a solid phase such, as a magnetic glass particle or silica surface, could have been subjected to a bisulfite treatment, as presently claimed. For example, it was generally known in the art at the time of the invention that the binding of single stranded DNA to a solid phase involves various interactions, including hydrogen bonds. In 2003, a person skilled in the art understood that the interactions between a single stranded DNA molecule and a solid phase were similar to the interactions between two, individual strands in a double stranded DNA molecule. Since bisulfite reacts only with unmethylated cytosines that do not participate in base pairing (*i.e.,* single stranded DNA), a person skilled in the art would have been surprised to learn that a bisulfite reaction could be accomplished using a

single stranded DNA bound to a solid phase. In fact, at the time the invention, my co-worker and I were also very surprised when we found out that the single stranded DNA bound to a solid phase could actually be accessed successfully by the bisulfite ions. The teachings of the above cited references would not have changed my surprise in this matter.

13. In summary, I submit that it would not have been obvious to a skilled worker at the time the Application was filed, who had knowledge of the above-cited art, to use solid phase bound denatured DNA for bisulfite treatment, as defined in the claims.

I hereby declare that all statements made herein are, to my own knowledge, true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the captioned patent application or any patent issued therefrom.

Date April 11, 2008

Christine Markert-Hahn, Ph.D.

Enclosures:
Curriculum Vitae
List of patents (applications)

Curriculum Vitae

Penzberg, April 3, 2008

Name:	Christine Markert-Hahn
Date and place of birth:	09.03.1960, Aschaffenburg, Germany
Marital status:	married, 2 children, born 1991 and 1994
Adress:	Saelweiherstraße 54, 82377 Penzberg
Education:	Abitur, Aschaffenburg, 1979 Diploma in Biochemistry, Tübingen 1985 PhD, Pettenkofer-Institut, Munich, 1989

Entry to Roche Diagnsostics GmbH (former Boehringer Mannheim): Nov 15th 1989

Position held at BM/ Roche: Manager in R&D Diagnostics 1989-2006

Working experiences at BM/Roche:

Rare reagent development, proteinchemistry 1989-1994

- Purification of antigens and antibodies for different product lines (Enzymun,. Elecsys, Point of Care) and indications
- Process establishement for conjugation of antigens and antibodies with different labels for different product lines (Peroxidase-, Biotin-, Ruthenium-conjugates)
- Transfer of purification and conjugation processes to manufacturing department
- Development of analytical methods, mainly immunoassys, for in process control

Molecular Diagnostics Infectious diseases 1995-1999

- Feasibility studies for DNA isolation from serum using magnetic glass particles (parameter HCV)
- System integration work for the SUBS-Project (single unit blood screening), main areas: DNA isolation, calibration, development of new algorithms for PCR evaluation (CobasTaqman Platform)

Assay development for Cobas Core and Elecsys 2000-2001

- Development of different autoimmunity assays according to DCC guidelines (RF = rheuma factor until LD, RFIgA and RFIgM until TTM) using the CobasCore Platform
- Feasibility study to transfer the ANAScreen Assay (Anti Nuclear Antibody) from CobasCore to Elecsys (DG)

Molecular Diagnostics Oncology 2002-2006

- Method establishment and method validation in the field of DNA methylation technology (DNA-Isolation, DNA-Modification, Decontamination)
- PCR-Optimization for marker validation (LC-Platform, COBAS Taqman-Platform)
- Clinical studies to validate markers

- IVD product development of a „Companion Diagnostic Assay“ based on gene expression analysis of different proteins in the HER-pathway (Reverse Transcriptase-PCR using the LightCycler)

Position held at BM/ Roche: Manager in Pharmaceutical Biotech Production since 2007

Experiences in Project-Management:

- From the beginning on internal project management according to current QM regulations (DCC) depending on the status of the projects worked on including MS-Project tool.

Leadership ability:

- Continuous responsibility for people for 15 years (R&D lab with 2-4 technician, and sometimes Azubi and PostDoc)
- Leading of interdisciplinary teams partly with external cooperants (Epigenomics) for several years
- Continuous improvement of personal leadership ability by special training courses

Scientific Publications:

- Lehmann HP, Block D, Markert-Hahn C, Zolg JW, "New concepts in systemic autoimmunity testing.", Scand J Clin Lab Invest Suppl. 2001;235:84-90;

- Markert-Hahn C, Berding C, Kuhn C., "Critical level and detection limit: performance measures for PCR-based assays.", J Virol Methods. 1998 Oct;74(2):139-48;

- Haberhausen G, Pinsl J, Kuhn CC, Markert-Hahn C., "Comparative study of different standardization concepts in quantitative competitive reverse transcription-PCR assays.", Clin Microbiol. 1998 Mar;36(3):628-33;

- Jilg W, Voltz R, Markert-Hahn C, Mairhofer H, Münz I, Wolf H., "Expression of class I major histocompatibility complex antigens in Epstein-Barr virus-carrying lymphoblastoid cell lines and Burkitt lymphoma cells.", Cancer Res. 1991 Jan 1;51(1):27-32

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Search results for: ((markert hahn christine) <in> IN)
Collections searched: US (Granted), German (Granted), German (Applications), European (Applications), European (Granted), INPADOC, Abstracts of Japan, WIPO PCT Publications, US (Applications)
62 matches found of 50,393,998 patents searched Displaying results **1 - 20** of **62**

Publication	Title	Pub. Date	Filed	Score
ES2290996T3	PROCEDIMIENTO PARA AISLAR UN ACIDO NUCLEICO.	2008-02-16	1998-09-29	77%
DK1394173T3	Forbedret fremgangsmåde til bisulfitbehandling	2008-02-04	2003-08-27	77%
DE60316642T2	Verbessertes Verfahren zur Bisulfit-Behandlung	2008-01-31	2003-08-27	77%
WO07068437A1	NEW METHOD FOR BISULFITE TREATMENT	2007-06-21	2006-12-12	77%
US20070190530A1	Method for bisulfite treatment	2007-08-16	2004-12-01	77%
JP2007175060A2	APPARATUS FOR ISOLATION OF ANALYTE IN BIOLOGICAL SAMPLE	2007-07-12	2007-02-27	77%
EP1829886A2	Improved method for bisulfite treatment	2007-09-05	2003-08-27	77%
EP1789586A2	DNA DECONTAMINATION METHOD	2007-05-30	2005-09-02	77%
EP1783135A1	Automatable method for preparing samples which can be universally applied	2007-05-09	1998-09-29	77%
EP1756313A2	SEQUENCE-SPECIFIC DETECTION OF METHYLATION IN BIOMOLECULES	2007-02-28	2005-06-14	77%
EP1394173B1	Improved method for bisulfite treatment	2007-10-03	2003-08-27	77%
EP1019430B1	Method for isolating a nucleic acid	2007-07-11	1998-09-29	77%
DK1019430T3	Fremgangsmåde til isolering af en nucleinsyre	2007-11-12	1998-09-29	77%
DE60316642C0	Verbessertes Verfahren zur Bisulfit-Behandlung	2007-11-15	2003-08-27	77%
DE59814056C0	Verfahren zur Isolierung einer Nukleinsaeure	2007-08-23	1998-09-29	77%
AT0374782E	VERBESSERTES VERFAHREN ZUR BISULFIT-BEHANDLUNG	2007-10-15	2003-08-27	77%
AT0366739E	VERFAHREN ZUR ISOLIERUNG EINER NUKLEINSAEURE	2007-08-15	1998-09-29	77%
WO06131391A1	PROGNOSTIC ASSAY FOR PREDICTION OF TREATMENT RESPONSE AND/OR SURVIVAL OF BREAST CELL PROLIFERATIVE DISORDER PATIENTS	2006-12-14	2006-06-09	77%
WO06084699A1	NEW METHYLATION MARKER	2006-08-17	2006-02-10	77%
WO06024541A3	DNA DECONTAMINATION METHOD	2006-03-09	2005-09-02	77%

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Publication	Title	Pub. Date	Filed	Score
WO06024541A2	DNA DECONTAMINATION METHOD	2006-03-09	2005-09-02	77%
US20060115907A1	Immune complex-specific antibodies for increased sensitivity in immunoassay array tests	2006-06-01	2005-10-28	77%
JP2006126202A2	IMMUNE COMPLEX SPECIFIC ANTIBODY FOR REDUCING BLANK VALUE IN ARRAY TEST METHOD, WHEN DETECTING ANTIGEN- SPECIFICALLY COMBINED ANTIBODY OF SPECIFIED IMMUNOGLOBULIN CLASS	2006-05-18	2005-10-28	77%
EP1692315A1	IMPROVED METHOD FOR BISULFITE TREATMENT	2006-08-23	2004-12-01	77%
EP1653233A1	Method for determining antibodies of a particular class using an immune complex-specific antibody	2006-05-03	2005-10-26	77%
DE102004052729A1	Immunkomplex-spezifsicher Antikoerper zur Reduktion des Nullwerts beim Nachweis von Antigen-spezifisch gebundenen Antikoerpern einer bestimmten Immunglobulinklasse in Array-Testformaten	2006-05-04	2004-10-30	77%
CA2526109AA	IMMUNE COMPLEX-SPECIFIC ANTIBODY FOR REDUCING THE BLANK VALUE IN ARRAY TEST FORMATS WHEN DETECTING ANTIBODIES OF A PARTICULAR IMMUNOGLOBULIN CLASS THAT HAVE BEEN BOUND IN AN ANTIGEN-SPECIFIC MANNER	2006-04-30	2005-10-28	77%
CA2576142AA	DNA DECONTAMINATION METHOD	2006-03-09	2005-09-02	77%
WO05121361A3	SEQUENCE-SPECIFIC DETECTION OF METHYLATION IN BIOMOLECULES	2005-12-22	2005-06-14	77%
WO05121361A2	SEQUENCE-SPECIFIC DETECTION OF METHYLATION IN BIOMOLECULES	2005-12-22	2005-06-14	77%
WO05054502A1	IMPROVED METHOD FOR BISULFITE TREATMENT	2005-06-16	2004-12-01	77%
CA2547743AA	IMPROVED METHOD FOR BISULFITE TREATMENT	2005-06-16	2004-12-01	77%
US20040241704A1	Method for bisulfite treatment	2004-12-02	2003-08-25	77%
JP2004089195A2	IMPROVED METHOD FOR BISULFITE TREATMENT	2004-03-25	2003-08-29	77%
EP1394173A1	Improved method for bisulfite treatment	2004-03-03	2003-08-27	77%
CA2438327AA	IMPROVED METHOD FOR BISULFITE TREATMENT	2004-02-29	2003-08-28	77%
US20030199078A1	Method, kit and apparatus for the isolation of nucleic acids	2003-10-23	2003-04-30	77%

US6562568	Method, kit and apparatus comprising magnetic glass particles for the isolation of biomolecules	2003-05-13	2000-09-05	77%
EP1019430A2	AUTOMATABLE METHOD FOR PREPARING SAMPLES WHICH CAN BE UNIVERSALLY APPLIED	2000-07-19	1998-09-29	77%
CZ20001132A3	AUTOMATED, UNIVERSALLY USABLE PROCESS FOR PREPARING SAMPLES	2000-08-16	1998-09-29	77%

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Publication	Title	Pub. Date	Filed	Score
WO9916781A3	AUTOMATABLE METHOD FOR PREPARING SAMPLES WHICH CAN BE UNIVERSALLY APPLIED	1999-09-16	1998-09-29	77%
WO9916781A2	AUTOMATABLE METHOD FOR PREPARING SAMPLES WHICH CAN BE UNIVERSALLY APPLIED	1999-04-08	1998-09-29	77%
ES2133436T3	CONJUGADOS INMUNOLOGICAMENTE ACTIVOS Y UN PROCEDIMIENTO PARA SU PREPARACION.	1999-09-16	1994-03-24	77%
ES2123677T3	LIGADORES DE MALEINIMIDA TRIFUNCIONALES HOMOBIDENTADOS Y SU USO EN CONJUGADOS INMUNOLOGICAMENTE ACTIVOS.	1999-01-16	1994-03-24	77%
EP0618231B1	Immunologically active conjugates and a method for their preparation	1999-06-02	1994-03-24	77%
DE59408322C0	IMMUNOLOGISCH AKTIVE KONJUGATE UND EIN VERFAHREN ZU IHRER HERSTELLUNG	1999-07-08	1994-03-24	77%
DE19743518A1	Automatisierbare universell anwendbare Probenvorbereitungsmethode	1999-04-15	1997-10-01	77%
CA2305171AA	AUTOMATABLE METHOD FOR PREPARING SAMPLES WHICH CAN BE UNIVERSALLY APPLIED	1999-04-08	1998-09-29	77%
EP0618192B1	Homobidental, trifunctional maleimide linkers and their application in immunologically active conjugates	1998-09-16	1994-03-24	77%
DE59406903C0	HOMOBIDENTALE, TRIFUNKTIONELLE MALEINIMID-LINKER, UND IHRE VERWENDUNG IN IMMUNOLOGISCH AKTIVEN KONJUGATEN	1998-10-22	1994-03-24	77%
AT0171163E	HOMOBIDENTALE, TRIFUNKTIONELLE MALEINIMID-LINKER, UND IHRE VERWENDUNG IN IMMUNOLOGISCH AKTIVEN KONJUGATEN	1998-10-15	1994-03-24	77%
US5601824	Homobidental, trifunctional linkers used in immunologically active conjugates	1997-02-11	1996-02-23	77%
US5573922	Immunological detection method for triazines	1996-11-12	1994-04-29	77%
US5519142	Homobidental, trifunctional linkers, method for their preparation and use in immunologically active conjugates	1996-05-21	1994-03-29	77%
US5514559	Immunologically active conjugates and method for their preparation	1996-05-07	1994-03-29	77%
EP0622633A3	Immunological detection of triazine	1995-10-11	1994-04-22	77%
EP0622633A2	Immunological detection of triazine	1994-11-02	1994-04-22	77%
EP0618231A1	Immunologically active conjugates and a method for their preparation	1994-10-05	1994-03-24	77%
EP0618192A1	Homobidental, trifunctional mazeimide linkers and their application in immunologically active conjugates	1994-10-05	1994-03-24	77%
DE4314091A1	Immunologisches Nachweisverfahren fuer Triazine	1994-11-03	1993-04-29	77%

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Publication	Title	Pub. Date	Filed	Score
DE4310142A1	Immunologisch aktive Konjugate und ein Verfahren zu ihrer Herstellung	1994-10-06	1993-03-29	77%
DE4310141A1	Homobidentale trifunktionelle Linker	1994-10-06	1993-03-29	77%

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